(M: 16±2%, n=6; F: 13±2%, n=15). BayK(-) increased flavoprotein oxidation in *mdx* cardiomyocytes from animals treated with a single weekly dose (M:13±3%, n=13; F: 10±3%, n=9) and with multiple doses per week (M: 17±2%, n=16; F: 14±2, n=24). These data indicate that either single or multiple i.p. injections per week of morpholino oligo peptides are effective at restoring cardiac mitochondrial function in both male and female *mdx* mice.

2940-Pos Board B370

High Susceptibility to Non-Alcoholic Fatty Liver Disease in Two-Pore Channel 2-Deficient Mice

Christian Grimm, Cheng-Chang Chen, Elisabeth Butz, Martin Biel, **Christian Wahl-Schott**.
Pharmacy, Pharmacology, LMU University of Munich, Muenchen, Germany.

Endolysosomal organelles play a key role in trafficking, breakdown and receptor-mediated recycling of different macromolecules such as low-density lipoprotein (LDL)-cholesterol, epithelial growth factor (EGF) or transferrin. In the liver, receptor-mediated uptake of low-density lipoproteins (LDLs) and subsequent intracellular transport is essential for hepatic cholesterol homoeostasis and plasma lipoprotein metabolism. Dysfunction within this pathway results in liver disease such as non-alcoholic fatty liver disease (NAFLD), which is associated with increased cardiovascular and liver-related mortality. It has been estimated that as many as 30% of adults in these countries have NAFLD. This liver disease has thus emerged as a substantial public health concern. Here, we examine the role of two-pore channel (TPC) 2, an endolysosomal cation channel, in these processes. Embryonic mouse fibroblasts were generated from wild type and TPC2 deficient mice and characterized by single lysosome patch clamp employing conventional patch clamp as well as planar patch clamp. In addition, trafficking assays were performed. Embryonic mouse fibroblasts and hepatocytes lacking TPC2 display a profound impairment of LDL-cholesterol and EGF/EGF-receptor trafficking. Mechanistically, both defects can be attributed to a dysfunction of the endolysosomal degradation pathway most likely on the level of late endosome to lysosome fusion. Importantly, endolysosomal acidification or lysosomal enzyme function are normal in TPC2-deficient cells. TPC2-deficient mice are highly susceptible to hepatic cholesterol overload and liver damage consistent with non-alcoholic fatty liver hepatitis. These findings indicate reduced metabolic reserve of hepatic cholesterol handling. Our results suggest that TPC2 plays a crucial role in trafficking in the endolysosomal degradation pathway and, thus, is potentially involved in the homoeostatic control of many macromolecules and cell metabolites.

Ion Channels, Pharmacology, and Disease

2941-Pos Board B371

Relevance of SARS-CoV E Protein Ion Channel Activity in Virus Pathogenesis

Carmina Verdiá Báguena[1], José L. Nieto-Torres[2], Marta L. De Diego[2], Jose M. Jimenez-Guardeño[2], Jose A. Regla-Nava[2], Raul Fernandez-Delgado[2], carlos Castaño-Rodríguez[2], Jaume Torres[3], Antonio Alcaraz[1], Vicente M. Aguilella[1], Luis Enjuanes[2].
[1]Department of Physics, Universitat Jaume I, Castellón de la Plana, Spain, [2]National Center of Biotechnology (CNB-CSIC), Campus Universidad Autónoma de Madrid, Madrid, Spain, [3]School of Biological Sciences, Division of Structural and Computational Biology, Nanyang Technological University, Singapore, Singapore.

Coronaviruses (CoV) are vertebrate pathogens that cause common colds, bronchiolitis and acute respiratory distress syndrome. In fact, their relevance increased when the causative agent of the severe acute respiratory syndrome (SARS) was identified as a CoV. CoV E protein is a small transmembrane protein of between 76-109 amino acids in length that modulates coronavirus morphogenesis, tropism and virulence [1].

We sought to elucidate the role of E protein IC activity in virus pathogenesis by combining our knowledge of residues essential for E protein ion conductivity with the manipulation of SARS-CoV genome. To test the contribution of E protein IC activity in virus pathogenesis, two recombinant mouse-adapted SARS CoVs, each containing one single amino acid mutation that suppressed ion conductivity, were engineered. After serial infections, mutant viruses, in general, incorporated compensatory mutations within E gene that rendered active ion channels. Furthermore, IC activity conferred better fitness in competition assays, suggesting that ion conductivity represents an advantage for the virus. Interestingly, mice infected with viruses displaying E protein IC activity, either with the wild-type E protein sequence or with the revertants that restored ion transport, rapidly lost weight and died. In contrast, mice infected with mutants lacking IC activity, which did not incorporate mutations within E gene during the experiment, recovered from disease and most survived.

We have shown that SARS-CoV E protein IC activity is a virulence determinant.

[1] DeDiego, M.L., et al 2008. Virology 376, 379-389.
[2] Verdiá-Báguena C., et al. 2012. Virology. 432: 485-494.

2942-Pos Board B372

Divalent Copper Compound as Inhibitory Agent of Influenza A

Kelly L. McGuire, Nathan A. Gordon, Roger G. Harrison, David D. Busath.
Physiology and Developmental Biology, BYU, Provo, UT, USA.

Influenza A virus (IAV) exhibits a high mutation frequency. Mutations in the proton channel M2 have created substantial IAV drug resistance to previously effective drugs, such as amantadine (AMT), along with its analogs. The main drug-resistant variation in the M2 proton channel, which has become ubiquitous in humans, is the mutation S31N. Divalent copper has previously been shown, using *in vitro* assays involving SSNMR and *Xenopus* oocytes, to bind and block wild type M2 at the His37 selectivity filter. Here we report initial tests of the hypothesis that, given the essential, conserved nature of the selectivity filter, a complex consisting of copper bound to an amantadine derivative might serve as a broad-spectrum anti-IAV drug. The EC_{50} of AMT, two AMT analogs, $CuCl_2 \bullet 2H_2O$, and four previously published Cu^{2+} complexes were tested for antiviral activity against the California/07/2009 (H1N1) IAV strain containing the S31N M2 proton channel in viral mini-plaque assays and for M2(22-62, S31N)-mediated proton transport block in liposomes. A novel, AMT-based divalent copper compound, NAG 107, emerged as a plausible lead with an EC_{50} of 2.91 ± 0.29 μM in the viral mini-plaque assay and 4.5 ± 0.6 μM in the liposome assay, 21-fold and 11-fold better than amantadine in these assays, respectively.

2943-Pos Board B373

Insights on Molecular Determinants of hERG K^+ Channel Inhibition: Design, Synthesis, and Biological Evaluation of Lubeluzole Derivatives

Roberta Gualdani[1], Maria Maddalena Cavalluzzi[2], Francesco Tadini-Buoninsegni[1], Maria Rosa Moncelli[1], Giovanni Lentini[2].
[1]Department of Chemistry, University of Florence, Sesto F.no (FI), Italy, [2]Department of Medicinal Chemistry, University of Bari, Bari, Italy.

Drug-induced block of 'Human ether-a-go-go-related Gene' (hERG) K^+ channels is the main reason of long QT syndrome, a disorder of cardiac repolarization which may lead to sudden death due to ventricular fibrillation. This side effect has been the most frequent cause for drug withdrawal from the pharmaceutical market. For this reason, understanding the molecular determinants of hERG channel inhibition is an interesting strategy to avoid the cardiotoxicity of drugs.

Lubeluzole, a neuroprotective compound, has been associated with the acquired long QT syndrome and ventricular arrhythmias; however its effects on the hERG K^+ channel have not been described to date. Thus, the molecular determinants for lubeluzole action on hERG channel were studied using the patch clamp technique. We found that lubeluzole and its enantiomer are highly potent inhibitors of hERG current with an IC50 value of around 10 nM (no stereoselectivity observed). Moreover we observed that hERG inhibition by lubeluzole is time- and voltage-dependent. In fact lubeluzole causes a negative shift in the voltage dependence of hERG current activation and accelerates tail current deactivation, suggesting that it preferentially blocks the activated state of the channel.

To go further in details, we synthesized a series of lubeluzole congeners designed to explore the role of lipophilicity and electronic distribution. Structural variations were designed on the basis of the indications gained by quantum-mechanical calculations run at several DFT levels.

For each compound we determined the relative hERG affinity and the state-dependent mechanism, to evaluate how specific modification of drug structure affected hERG binding interaction. Main SAR results will be presented.

2944-Pos Board B374

Validation of KCa3.1 Channel Nifedipine Interaction Site Predicted by Rosetta Modeling Method

Hai M. Nguyen[1], Heike Wulff[1], Vladimir Yarov-Yarovoy[2].
[1]Pharmacology, University of California, Davis, Davis, CA, USA, [2]Physiology and Membrane Biology, University of California, Davis, Davis, CA, USA.

Calcium-activated potassium channels, including the intermediate-conductance KCa3.1 channel and the related small-conductance KCa2 channels,